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# Synthesis and Antibacterial Studies of New 1,2-Bis(Carbonoylthiourea)-Propane

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***Abstract :*** 1,2-bis(benzoylthiourea)-propane, 1,2-bis(butyrylthiourea)-propane, and 1,2-bis(4-chlorobenzoylthiourea)-propane were successfully synthesized in the reaction of benzoyl-, butyryl- and 4-chlorobenzoyl-isothiocyanate with 1,2-diaminopropane. The compounds were characterized by infra red and nuclear magnetic resonance spectroscopic techniques. All three compounds showed antibacterial activities against *Bacillus subtilis* and *Salmonella typhymurium*, with inhibition zone range of 8.67-12.00 mm. The inhibition zone for tetracycline control is 15.00 mm.

*Keywords:* 1,2-bis(carbonoylthiourea)-propane; aroyl-isothiocyanate; 1,2-diaminopropane; antibacterial activity

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## Introduction

Thiourea and its derivatives have been used in many fields of science and technology. The compounds are important for electrodeposition of metals [1], rubber vulcanization, agricultural industry [2,3], veterinary, textile treatments [4] and food science [5,6] Compounds of thiourea have also shown biological activities [7,8] and therefore are potential candidates for pharmaceutical and drugs [9,10]. The potent biological activities displayed by several thiourea derivatives have led to comprehensive efforts in preparing and evaluating the biological activities of substituted thioureas [11]. In our continuing effort to synthesize new carbonoyl thiourea derivatives, three compounds namely 1,2-bis(benzoylthiourea)-propane, 1,2-bis(butyrylthiourea)-propane and 1,2-bis(4-chlorobenzoylthiourea)-propane were obtained. The structural characterization of the synthesized compounds were made by elemental analysis, infra red and nuclear magnetic resonance spectroscopic techniques. The compounds exhibited antibacterial activities against Gram-positive bacteria: *Bacillus subtilis* and Gram-negative bacteria: *Salmonella typhymurium*, by using the disc diffusion method.

## Experimental

### *Materials and methods*

All the chemicals were reagent grade quality obtained from Aldrich E, Merck and BDH. They were used without further purification. The microelemental CHN-S data were performed using a Thermo Finnigan Flash Model EA 1112 Series. Fourier Transform Infra Red (FT-IR) spectrum in a range of 4000 $cm^{-1}$ to 400 $cm^{-1}$ was recorded using KBr pellet on a Perkin Elmer Model Spectrum GX apparatus. Nuclear magnetic resonance (NMR) of $^{1}H$ and $^{13}C$ were obtained in $d_6$ DMSO solutions on a JEOL EX400 instrument. Melting points were determined using Electrothermal 9300 Digital Melting Point Apparatus.

### *Synthesis*

A solution of 1,2-diaminopropane, 0.01 mol in acetone (20 mL) was added dropwise to an acetone solution (20 mL) containing equimolar amounts of benzoylchloride (0.02 mol) and ammonium thiocyanate (0.02 mol). The mixture was refluxed for 1.5 h. The resulting solution was poured into a beaker containing ice cubes. The white precipitate was filtered off, washed with distilled water and ethanol, and then dried at room temperature. The above reaction was repeated with butyrylchloride and 4-chlorobenzoylchloride.

### *Antibacterial Test*

The disc diffusion test was done based on the method by Abdelhalim *et al.* [12] with some modifications on the preparation of test compounds. The method included the preparation of inoculum, test compounds and the inoculation onto agar.

### *Preparation of Inoculum*

The microrganisms used were *Salmonella typhymurium, Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*. The isolation of these

bacteria were cultured in Mueller Hinton Broth (MHB) and incubated at 37 °C for 24 h. The turbidity of bacterial growth was measured by biophotometer at OD 600 [13].

*Preparation of Test Compounds and Antibiotic*

5 mg of each test compounds was dissolved in 1 mL of dimethylsulfoxide (DMSO) and 9 mL of diluted water separately to prepare solution with the concentration of 500 µg/mL. While the antibiotic of tetracycline was already prepared and taken from the Microbiology Lab FST USIM. All the disc diffusion and dilution of compounds were performed in duplicate and repeated two times.

*The Inoculation of Bacteria and Test Compounds*

The 0.1 mL of freshly prepared bacterial cells was spread onto Mueller Hinton agar (MHA) plate using hockey stick. The impregnated disc (5 mm) containing test compounds and tetracycline were each placed on the surface of the agar inoculated with the bacteria. The plates were incubated at 37 °C for 24 h. The invisible growth on the plates was recorded. DMSO will be used as a solvent control to ensure that solvent had no effect on bacterial growth. Tetracycline was designated in this experiment as a control drug [12].

**Results and discussion**

*Characterization*

Three new bis-thiourea derivatives namely 1,2-bis(benzoylthiourea)-propane (**A)**, 1,2-bis(butyrylthiourea)-propane (**B**) and 1,2-bis(4-chlorobenzoylthiourea)-propane (**C**) were successfully synthesized. The microelemental analysis data of the compounds are in agreement with the calculated values. Analytical data and some physical properties of 1,2-bis(carbonoylthiourea)-propane are listed in Table 1.

**Table 1 :** Analytical and physical data for 1,2-bis(carbonoylthiourea)-propane

| Structural formula | Yield (%) | Mp (°C) | Element (%) | | | |
|---|---|---|---|---|---|---|
| | | | C | H | N | S |
| $[C_6H_5CO(NH)CS(NH)]_2$ $CH_2CH(CH_3)$ , **A** | 79.95 | 107-109 | 57.18 (57.29) | 4.89 (4.52) | 15.17 (14.07) | 17.52 (16.08) |
| $[CH_3CH_2CH_2CO(NH)CS(NH)]_2$ $CH_2CH(CH_3)$, **B** | 78.23 | 106-109 | 47.85 (46.99) | 7.16 (7.23) | 17.67 (16.87) | 18.91 (19.28) |
| $[C_6H_4ClCO(NH)CS(NH)]_2$ $CH_2CH(CH_3)$, **C** | 79.70 | 108-110 | 49.23 (48.61) | 3.93 (3.84) | 12.27 (11.94) | 12.25 (13.65) |

$^1$H NMR spectra showed the presence of three important chemical shifts in all the compounds: amide amino proton δH(CONH) 11.15-11.38 ppm, thioamide amino proton δH(CSNH) 10.66-10.77 ppm and δH(Ar-H) at 7.49-7.90 ppm (Table 2).

**Table 2 :** $^1$H NMR spectral data (δ, ppm) of 1,2-bis(carbonoylthiourea)-propane in $d_6$ DMSO (s=singlet, d=dubel, m=multiplet)

| Compound | δH(CONH) | δH(CSNH) | δH(Ar-H) |
|---|---|---|---|
| **A** | 11.30 (d, 2H) | 10.87 (d, 2H) | 7.49-7.90 (m, 10H) |
| **B** | 11.15 (d, 2H) | 10.66 (d, 2H) | - |
| **C** | 11.38 (d, 2H) | 10.77 (d, 2H) | 7.55-7.89 (m, 8H) |

Meanwhile, $^{13}C$ NMR spectra showed that the thione, C=S and ketone, C=O chemical shifts' were observed at 180.10-181.15 ppm and 166.33-175.20 ppm respectively in all the three compounds (Table 3).

**Table 3 :** $^{13}C$ NMR spectral data (δ, ppm) of 1,2-bis(carbonoylthiourea)-propane in $d_6$ DMSO

| Compound | δ(C=S) | δ(C=O) |
|---|---|---|
| **A** | 180.19,181.14 | 167.98,168.11 |
| **B** | 180.10,181.10 | 175.15,175.20 |
| **C** | 180.18,181.15 | 166.33,167.28 |

FTIR spectra of all the compounds exhibited the presence of ν (N-H) , ν (C=O) , ν (C=S) $cm^{-1}$ and ν (C-N) stretching frequencies at 3163.43-3188.38 $cm^{-1}$, 1672.62-1694.41 $cm^{-1}$, 737.55-765.58 $cm^{-1}$ and 1094.12-1186.67 $cm^{-1}$ respectively as shown in Table 4.

**Table 4 :** Major FTIR absorption bands ($cm^{-1}$) of 1,2-bis(carbonoylthiourea)-propane (s=strong, m=medium, w=weak)

| Compound | ν (N-H) | ν (C=O) | ν (C=S) | ν (C-N) |
|---|---|---|---|---|
| **A** | 3163 s | 1676 w | 765 m | 1163 m |
| **B** | 3188 s | 1694 w | 737 m | 1186 m |
| **C** | 3173 s | 1672 w | 757 m | 1094 m |

*Antibacterial Activity*

The in vitro antibacterial activity of the bis-thiourea derivatives **A, B** and **C** against two strains of Gram-positive bacteria and two strains of Gram-negative bacteria were investigated in comparison to tetracycline (control). Table 5 listed the results of antibacterial activities of the tested compounds.

**Table 5:** Diameter zone of inhibition (mm) for antibacterial test of 1,2-bis(carbonoylthiourea)-propane using the disc diffusion method

| **Compound** | **Concentration (µg/mL)** | **Diameter (mm)** | | | |
|---|---|---|---|---|---|
| | | ***S. aureus*** | ***B. subtilis*** | ***E. Coli*** | ***S. typhimurium*** |
| **A** | 500 | a- | - | - | 10.2 ± 1.9 |
| **B** | 500 | - | 9.0 ± 0.8 | - | 8.7 ± 0.6 |
| **C** | 500 | - | 9.3 ± 0.6 | - | 12.0 ± 0.0 |
| DMSO | - | - | - | - | - |
| Tetracycline | Neat sample | 40.0 ± 0.0 | 15.0 ± 0.0 | - | - |

* DMSO= dimethylsulfoxide; a- = no zone of inhibition

According to Ozmen and Olgun [14], the presence of electron densities on donor atom such as oxygen, sulphur and nitrogen in compounds contributed positively to the activity of compounds against bacteria. From Table 5, the results revealed that all the three tested compounds were found to have antibacterial effects against *S. typhimurium* and two of them exhibited an inhibition of the growth of *B. subtilis.* Compound A only showed good activity against Gram-negative bacteria: *S. typhimurium*, whereas compounds B and C exhibited moderate effectiveness towards *B. subtilis* and *S. typhimurium.* However, all the tested compounds were inactive against *E. coli* and *S. aureus*.

Among the tested compounds of A and C, the inhibitory effect appears to be dependent on the substitution at the benzene ring. The presence of a chloro atom in the benzene ring of compound C, improved antibacterial activity in respect to compound A. Thus, chloro derivative is generally more effective against the tested microorganism and acts as the most potent inhibitors of the growth of some microorganisms as *S. typhimurium* and *B. subtilis.*

It is interesting to point out that the antibacterial activity of the tested compounds against *S. typhimurium* is more effective than against *B. subtilis*, concluded that the compounds showed stronger antibacterial activity against Gram-negative than Gram-positive bacteria. This observation may be attributed to their different cell walls [15]. *S. typhimurium* is a typical Gram-negative bacteria, the cell wall of which is made up of thin membrane of peptidoglycan and an outer membrane constituted of lipopolysaccharide, lipoprotein, and phospholipids. The thiourea compounds disrupted the barrier properties of the outer membrane of Gram-negative bacteria. This is due to the presence of C=O, C=S, and NH groups in thiourea derivative compounds that can be protonated under acidic conditions. Thus, they react with the carboxyl and phosphate group of bacterial surface and therefore show antibacterial activity against Gram-negative bacteria. On the other hand, Gram-positive bacteria: *B. subtilis* has cell wall composed solely of peptidoglycan, which does not allow the formation of a surface layer [15].

## Conclusions

In this study, we have reported the synthesis of new 1,2-bis(carbonoylthiourea)-propane derivatives. The structural characterizations of the synthesized compounds were made by using the elemental analysis and spectroscopic methods. All the three tested compounds exhibited varying degrees of inhibition effects on the growth of the selected bacteria.

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